Comparative Effect of Honey and Antibiotics against Multi Drug Resistant Bacteria Isolated from Surgical Site Infection

Syeda Rahmat Bibi¹, Zobia Afsheen¹, Hamza Iftikhar³, Ranra Jalal², Saad Jan³, and Syed Majid Rasheed³*

¹Department of Microbiology and Biotechnology, Abasyn University, Peshawar, Pakistan
²Department of Weed Sciences and Botany, University of Agriculture Peshawar, Pakistan
³Department of Agriculture, Bacha Khan University, Charsadda, Pakistan

Abstract: Patients undergoing surgery are predominantly exposed to surgical site infections (SSI) resulting in serious consequences. A cross-sectional study comprising 100 samples, was collected from various surgical sites of admitted and non-admitted patients in Lady Reading Hospital, Peshawar. 87 samples were tested positive for bacterial growth. After isolation and identification, the highest prevalence was recorded among isolates of Staphylococcus aureus 38 (43.6%) followed by Pseudomonas sp. 25 (28.7%), Streptococcus pyogenes 13 (14.9%) and E. coli 11 (12.6%). Antibiogram pattern was determined which showed high sensitivity of all bacterial isolates toward Clindamycin, Clarithromycin, and Piperacillin, followed by Erythromycin, Doxycycline, Co-amoxiclav, while maximum number of isolates showed resistance against Vancomycin, Ciprofloxacin, Amikacin. According to quantitative analysis, Ziziphus and Acacia honey inhibited E. coli at different Minimum Inhibitory Concentration (MIC). The results revealed that Ziziphus honey inhibited E.coli at 100 µl concentration on lowest MIC at 0.25 µl while Acacia honey inhibited E.coli at 75 µl concentration on lowest MIC at 0.5 µl. Ziziphus honey inhibited Pseudomonas sp. at 100 µl concentration on lowest MIC at 0.25 µl while Acacia honey inhibited Pseudomonas at 100 and 75 µl concentration on lowest MIC at 1 µl. Ziziphus honey also inhibited Staphylococcus aureus at 100 and 75 µl concentration on lowest MIC at 0.25 µl, while Acacia honey inhibited S. aureus at 100µl concentration on lowest MIC at 0.5 µl. This study concludes that Ziziphus honey was more effective in curing surgical wounds compared with Acacia honey. However, further Studies needed to be done for Ziziphus honey to utilize it as an efficient treatment approach.

Keywords: Surgical Site Infection, Antibiotics, Acacia, Ziziphus, Bacterial Isolates.

1. INTRODUCTION

Skin is a natural barrier against infection. The most important function of skin is to inhibit microbes that reside in the skin’s superficial layer and minimize the proliferation of pathogens from invading underlying tissues. Although there are many protocols and precautions which are responsible for preventing infection but exposure of subcutaneous tissues to certain bacteria easily destroys skin’s structure, thus provides a nutritious, warm, and moist environment that is favorable to microbial proliferation and colonization. Surgical Site Infections (SSIs) are the most important cause of the accumulation of flora at or near the surgical site. In contaminated surgery, urinary, intestinal genital, and respiratory flora also infect the site. Physicians name these infections as SSIs because they occur in body parts that have undergone surgery. As described by Centers for Disease Control and Prevention (CDC) three different kinds of SSIs occur which include superficial incisional SSI, deep incisional SSI and organ or space SSI. SSIs reveal signs and symptoms such as fever, redness, swelling accompanied by tenderness, pain warmth and pus [1].
SSIs are one of the most common complications for patients undergoing surgical procedures and the second most frequently occurring healthcare-associated infection (HAI) [2, 3]. Due to SSIs, most of the patients are readmitted to hospital after surgery [4]. Initial infections, develop within one week of surgery, are frequently more serious [5]. Infections after surgery are caused by microorganisms. Microorganisms may invade an operational wound by different means of contact, such as touching a contaminated caregiver or surgical tools, aerosols, or microbes that are already on or in your body and then spread into the wound. Several bacterial species were isolated from wounds which include *Streptococcus*, *Staphylococcus* and *Pseudomonas*; however, *Staphylococcus aureus* was the most abundant species among wound isolates [6]. In severe wounds and burned cases, *Pseudomonas aeruginosa* is mostly isolated [7]. Methicillin-resistant *Staphylococcus aureus* causes 37% of SSI infections in community hospitals [8].

After surgery, SSIs can still occur within 30 days and may have a chance to occur within 1 year in patients who experienced implantation during surgery. Majority of SSIs, almost 12.84% are first diagnosed after the discharge of patient from the hospital. The risk of SSI is decreased by the use of short doses of antimicrobial drugs. Selection of antimicrobial agents depend on the pathogens, usually related to protocols being performed. Broad spectrum β lactam antibiotics are frequently used during surgical site preparation, and in order to kill infection causing anaerobes Metronidazole is used if needed and Vancomycin is not suggested for routine prophylaxis. The primary dose should be given at appropriate time to confirm that bactericidal values are present in tissue and serum at the incision time and necessary to maintain bacterial amounts for few hours after stitching wound in the operation theatre [9].

Antibiotics inhibit bacterial infections, but unfortunately, the efficacy of drugs is reduced with the passage of time because of increasing use of drugs especially different generations of antibiotics. Manufacturing novel antibiotics is difficult as huge financial expenses are required to test them by keeping in view the side effects which may occur after drug use. During recent years, health care professionals investigated high number of infections due to strains resistant to some antibiotics mostly because of drugs abuse. Natural resources have regained their preference as the primary alternatives for treating SSIs. Between these are the bee hive products such as honey, traditionally regarded as a very effective non-toxic material with antimicrobial properties and wide range of health benefits. Honey was considered to be used for medical and health purposes since 2000 BC. Currently, the use of honey to treat wounds is widely considered [10].

Honey is the natural sweet substance extracted from parts of plants which has been collected by honey bees (*Apis mellifera*) and then stored by them in the hives for future use [11]. However, in traditional medicine, honey is widely used, whereas in modern medicine it is inadequate [12]. Honey is a treatment option for many illnesses and frequently used for dressing surgical wounds, burns, and skin ulcers. It activates the growth of new tissues and heals wounds; it is also a pain reliever and eliminates odor [13]. The enzymes present in honey are the major factors due to which it is useful to human health. Honey is composed of three key enzymes, i.e., diastase (amylase), invertase (saccharase) and glucose oxidase [14]. In raw honey, glucose oxidase is not activated instead shows activity upon dilution with wound lesions. The enzymatic activity in honey results in production of hydrogen peroxide. Honey’s inherent antiseptic properties render it exceptionally valuable for various applications. Its optimal antibacterial action is achieved when used within the range of 30−50%, surpassing the efficacy of conventional drugs typically employed to treat urinary tract infections [15]. Honey has healing properties, it heals the wound by making it moist and rapidly treats infection, prevents and decrease exudation, edema and inflammation. It causes abrupt activation of angiogenesis, granulation and epithelialization, thus making the healing process more rapid [16]. Honey exhibits antimicrobial action because of its pH, osmolarity and production of hydrogen peroxide and due to presence of phytochemical components, e.g., methylglyoxal [17].

The current study was designed to diagnose the root cause of surgical site infection and drug resistance patterns of different isolates for facilitating the medical experts to select empirical antimicrobial therapy. The recent nosocomial infection scenario revealed the emergence of multi-
drug resistant bacteria, so potential therapeutic agents are needed to control, eradicate and investigate these resilient pathogens. An initial in vitro evaluation of honey originating from *Acacia modesta* and *Zizyphus jujube* was also described in previous studies [18].

The aim of the proposed study was to determine antibiogram of honey from *acacia* and *ziziphus* compared with commonly used antibiotics against multi drug resistant (MDR) bacteria isolated from surgical site infections using broth dilution and spectroscopic technique.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Collection and processing of hundred surgical wound samples were carried out aseptically using cotton swabs which were carefully sterilized. Samples were collected from patients of both genders aged >24 years, visiting Lady Reading Hospital (LRH), Peshawar. After collection, samples were transported to the Microbiology Research Laboratory (MRL), Abasyn University Peshawar.

2.2 Sample Inclusion and Exclusion Criteria

Collection of samples was carried out from the wounds of surgical sites, from pus discharge in surgical wounds along with serous or sero-purulent discharge, and untreated sepsis patients. A complete history of sex, age, type of illness, diagnosis, the associated co-morbid diseases and type and duration of surgery performed, were obtained from the patients except those patients already using antibiotics, were included in the history.

2.3 Bacteriological Assessment of Microorganisms Present in the Wound Samples Colony Morphology

The sample was cultured on MacConkey’s agar, nutrient agar, and blood agar plates and incubated for 24 hours at 37 °C. After incubation, pure culture study (cultural characteristics, Gram staining, and different biochemical) using standard operating procedures was performed to identify bacterial isolates.

2.4 Microscopy/Gram Staining

Standard Gram staining procedure was performed for microscopy, smear of bacterial isolates was prepared and heat stabilized on clean slides. Two drops of crystal violet were applied on smear and washed with distilled water after 2 min. The second stage involved staining Smear and Gram’s iodine for 45 seconds before washing it with distilled water. The smear was exposed to decolorized 95% ethyl alcohol for 15 seconds in the following stage. Safranin was then used to cover the smear before being rinsed. Afterwards, all the slides were examined under the oil emulsion objective lens (100X).

**Table 1.** Colony features, gram stain reaction, and biochemical tests for identification of bacteria.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Colony Features</th>
<th>Gram Reaction</th>
<th>Coagulase</th>
<th>Oxidase</th>
<th>H₂S</th>
<th>Indole Test</th>
<th>Citrate Test</th>
<th>Urease Test</th>
<th>Catalase Test</th>
<th>TSI Test</th>
<th>Identified Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Large, opaque, flat colonies with irregular margins showing greenish coloration</td>
<td>-ve Rods</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A/A</td>
<td>P. aeruginosa</td>
</tr>
<tr>
<td>2.</td>
<td>Thick, greyish white, moist, smooth, opaque</td>
<td>-ve Rods</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>A/AG</td>
<td>E. coli</td>
</tr>
<tr>
<td>3.</td>
<td>Round, smooth, raised, gray to deep golden yellow</td>
<td>+ve Cocci</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>A/A</td>
<td>S. aureus</td>
</tr>
<tr>
<td>4.</td>
<td>Round, raised, shiny, gray, and have complete edges</td>
<td>+ve Cocci</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>A/A</td>
<td>S. pyogenes</td>
</tr>
</tbody>
</table>

Key: ‘A’ = Acidic., ‘Alk’ = Alkaline., AG’ = Acid and Gas., ‘-’ = Negative ‘+’ = Positive ‘+/-’ = May show negative or positive
2.5 Biochemical Assessment

Gram +ive and Gram -ive isolates were recognized by using different biochemical tests, i.e., urease test, Coagulase, Triple Sugar Iron (TSI) test, catalase, Citrate test, Oxidase test and Indole test (Table 1).

2.6 Antibiotic’s Susceptibility Profile against Isolated Bacteria

Kirby Bauer disc diffusion method was implemented to detect antibiotic susceptibility patterns. The isolates were subculture on Muller Hinton Agar (MHA) plates. Results of susceptibility pattern were determined according to guidelines of Clinical Laboratory Standards Institute, 2019 [19].

2.7 Antimicrobial Sensitivity Testing

The entire 4 isolates were tested against 10 antibiotics, i.e., Co-amoxiclav (10 µg), Vancomycin (30 µg), Erythromycin (15 µg), Cephradine (30 µg), Clindamycin (30 µg), Clarithromycin (30 µg), Ciprofloxacin (5 µg), Cefotaxime (30 µg), Doxycycline (30 µg), Gentamicin (10 µg), Amikacin (30 µg) and Piperacillin/Sulbactam (30 µg) to detect MDR strains by following Agar disc diffusion sensitivity method as mentioned in the guidelines of the National Committee for Laboratory Standards (NCLS) [20].

2.8 Determination of Minimum Inhibitory Concentration (MIC)

To determine MIC of different brands of honey against isolated bacteria broth dilution method was used. For this purpose, bacterial cultures containing broth media were transferred to sterile tube. To these culture tubes, different concentrations of honey were added. One test tube was left containing media and test organism as control. In shaking incubator, all the tubes were incubated at 37 °C and 120 rpm for 12 hours. Following that, MIC was calculated by using a spectrophotometer to measure optical density (OD) values at 610 nm [21].

2.9 Antibacterial Activity of Honey against Isolated Bacteria

A screening well diffusion test was conducted with some alterations inoculating nutrient agar plates (Oxford, U.K.) by rubbing sterile cotton swabs that were dipped into bacterial suspensions (on nutrient agar cultures grown at 37 °C and adjusted to 0.5 McFarland in sterile saline for 12 hours) above the whole surface of the plate. After incubation using a sterile cork borer 8.2 mm diameter wells were bored into the agar surface. Test honey of about 100 µl was poured into every well. Then these plates were incubated at 37 °C for 24 h and plates in which P. aeruginosa were present were incubated at 30 °C. Methylene blue was used for diffusion control with the help of Vernier caliper (Draper). The zones of inhibition were found out with a scale. The diameter of the well and diameter of the zone was measured. Each assay was carried out in triplicate.

3. RESULTS

In the current research study, a total of 100 samples were collected from different patients having surgical site infections, visiting Leady Reading Hospital, Peshawar. Samples were collected from both male and female patients (Table 2). Out of 100 collected samples, 87 samples were found positive for bacterial growth, whereas 13 were found negative among positive samples, bacteria were identified based on Gram staining, colony morphology, its general characteristics and biochemical tests. The frequency of identified isolates is given in Table 3.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Total number of samples of SSI</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>43</td>
<td>39</td>
</tr>
<tr>
<td>Female</td>
<td>57</td>
<td>48</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Isolates</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Staphylococcus aureus</em></td>
<td>38</td>
<td>43.7%</td>
</tr>
<tr>
<td>2.</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>25</td>
<td>28.7%</td>
</tr>
<tr>
<td>3.</td>
<td><em>Streptococcus pyogenes</em></td>
<td>13</td>
<td>15.0%</td>
</tr>
<tr>
<td>4.</td>
<td><em>Escherichia coli</em></td>
<td>11</td>
<td>12.6%</td>
</tr>
</tbody>
</table>
3.1 Antibiogram Analysis of Bacterial Isolates

Antibiotic susceptibility was performed on Muller Hinton Agar media using 12 different antibiotics. The results showed high sensitivity of all bacterial isolates toward Clindamycin, Clarithromycin, Piperacillin, Erythromycin, Doxycycline, and Co-amoxiclav, while maximum number of isolates showed resistance against Vancomycin, Ciprofloxacin, and Amikacin as shown in Table 4. The isolated bacterial species of *Staphylococcus aureus* were tested for antibiotics sensitivity profile, Clindamycin, Clarithromycin, and Cephradine showed highly sensitivity against *S. aureus* and Ciprofloxacin, Amikacin and Co-amoxiclav showed highly resistant. The isolated bacterial species of *Pseudomonas aeruginosa* were tested for antibiotics sensitivity profile. Clindamycin, Clarithromycin, and Cephradine showed high sensitivity and Ciprofloxac, Vancomycin, and Amikacin are highly resistant. The isolated bacterial species of *Streptococcus pyogens* were tested for antibiotics sensitivity profile. Piperacillin, Erythromycin, and Clindamycin showed high sensitivity, and Vancomycin, Ciprofloxac, and Amikacin are highly resistant. The isolated bacterial species of *Escherichia coli* were tested for antibiotics sensitivity profile. Amikacin, Clindamycin, and Co-amoxiclav showed high sensitivity and Vancomycin Ciprofloxac, and Cephradin are highly resistant.

### Table 4. Antibiotic susceptibility pattern of bacterial species.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>S. aureus</th>
<th>P. aeruginosa</th>
<th>S. pyogens</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>22</td>
<td>16</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>23</td>
<td>15</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>16</td>
<td>22</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Amikacin</td>
<td>12</td>
<td>26</td>
<td>09</td>
<td>16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>29</td>
<td>09</td>
<td>07</td>
<td>18</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>25</td>
<td>13</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Cephradine</td>
<td>26</td>
<td>12</td>
<td>19</td>
<td>6</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>27</td>
<td>11</td>
<td>21</td>
<td>4</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>29</td>
<td>09</td>
<td>20</td>
<td>5</td>
</tr>
<tr>
<td>Ciprofloxac</td>
<td>5</td>
<td>33</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>31</td>
<td>7</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>25</td>
<td>13</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>
3.4 Minimum Inhibitory Concentrations (MIC) Assays of Honey against Bacterial Isolates

The quantitative analysis of *Ziziphus* and *Acacia* honey (Figure 2(a)) showed that *Ziziphus* and *Acacia* inhibited *E. coli* at different concentrations. The results showed that *Ziziphus* honey inhibited *E. coli*, at 100 mg/ml concentration and showed lowest...
MIC result of 0.25 µl. *Acacia* honey inhibited *E. coli* at 75 mg/ml concentration low MIC at 0.5 µl. The results revealed that *Ziziphus* honey inhibited *Pseudomonas* at 100 mg/ml, 75 mg/ml concentration on the lowest MIC result is 0.25 µl (Figure 2(b)). *Acacia* honey inhibited *Pseudomonas* at 100 mg/ml and 75 mg/ml concentration on low MIC is 1 µl. It is revealed from Figure 2(c) that *Ziziphus* honey inhibited *S. aureus*, at 100 mg/ml and 75 mg/ml concentration on lowest MIC at 0.25 µl. *Acacia* honey inhibited *S. aureus* at 100 mg/ml concentration on the lowest MIC at 0.5 µl. The results from Figure 2(d) show that *Acacia* honey inhibited *S. pyogenes*, at 100 mg/ml and 75 mg/ml concentration on lowest MIC at 0.25 µl. *Ziziphus* honey inhibited *S. pyogenes* at 100 mg/ml and 75 mg/ml concentration on lowest MIC at 0.5 µl, respectively.

4. DISCUSSION

Surgical Site Infections are one of the most common complications for patients undergoing surgical procedures and also the second most frequently occurring healthcare-associated infection (HAI) [22, 23]. SSIs led to increased readmission cases in hospitals, increased morbidity, and mortality, reoperation, and prolonged hospital stays which exceeded the health care expenses and may result in significant production of drug-resistant bacterial species [24-26]. Initial infections are frequently more serious, and develop within one week of surgery [5], infections after surgery are caused by microorganisms. Microorganisms may invade an operational wound by different means of contact, such as touching, contaminated care providers or surgical tools, aerosols, or through microbes that are already on or in your body and then spread into the wound [6].

In the present study, a total of 100 samples collected from Surgical Site Infection (SSI) were processed for bacteriological analysis. Out of 100 collected samples, 87 (87%) were positive and showed presence of *S. aureus*, *P. aeruginosa*, *S. pyogenes*, and *E. coli*, while 13 (13%) were negative. The bacterial isolates were identified with *Staphylococcus aureus*, 38 (43%), *Pseudomonas aeruginosa* 25 (28.7%) followed by *Streptococcus pyogenes* 13 (15%), and *E. coli* 11 (12.6%), respectively. According to Bhattacharya *et al.* [27] total of 3004 cases of SSI were considered in which bacterial isolates frequency was as followed: *S. aureus* (34.93%), *E. coli* (20.34%), *Klebsiella spp* (18.08%) *Pseudomonas* (7.99%), respectively. The difference in the identified flora and their respective frequencies of Bhattacharya *et al.* [27] finding in comparison with our research findings might be due to the predominance of those particular microorganisms in that particular locality.

In our study out of total 100 samples, 43 were taken from male and 57 were from female patients. Among 43 samples from male patients, 39 (44.8%) were positive, while out of 57 female patients, 48 (65.5%) were positive while in a similar study conducted by Bhattacharya *et al.* [27], the findings of this parameter revealed 62.54% male and 37.45% female were positive. This difference in gender wise positivity of their comparative studies might be due to a large population size of the later researcher.

In the current study, out of 87% positive samples, the site wise distribution was as follow: Cholecystectomy (90%), Appendectomy (93.7%), Diabetic Toe Amputation (80%), Diabetic Foot Amputation (89.2%), and Herniotomy (84.6%). These findings are in contradiction with the results of Bhattacharya *et al.* [27] who collected samples from different surgical wards including: Surgery (12.49%), Orthopedics (11.85%), Urology (3.67%) & Pediatrics Surgery (2.25%) instead of particular surgical sites.

Commercially available antibiotics were evaluated in current study for their antimicrobial activity against the identified bacterial isolates. The results of the experiments revealed that most potent antibiotics found against all the tested bacterial isolates were: Clindamycin Clarithromycin, Piperacillin, and Cefotaxime, Co-amoxiclav, Vancomycin, and Cephradine, respectively, while maximum number of bacteria displaying resistance against Gentamicin, Ciprofloxacin and Amikacin. Our findings regarding antibiotics profiling against Co-amoxiclav are similar to those of Abubakar [28], who also found Co-amoxiclav as the most sensitive of all tested antibiotics in his study. The findings of Bhatt *et al.* [29] are in agreement with our studies in terms of evaluating the potency of commercially available antibiotic against isolated specimen. The tested bacteria displayed highest resistance to Gentamycin and Amikacin and displayed sensitivity
to Piperacillin antibiotic in their study. The most possible reason for the difference in sensitivity profile might be the improper use of antibiotics, over dosage or self-medication. Therefore, proper and prescribed dosage of medicine was recommended along with the maintenance of good hygienic conditions within hospitals as well as in routine life. In addition, the antibiotics must be used after performing susceptibility tests. Following these protocols might reduce the phenomenon of antibiotic resistance to a greater extent.

In the current study, *Acacia* honey and *Ziziphus* honey were tested for their antibacterial activity against *S. aureus*, *S. pyogenes*, and *E. coli*, at different concentrations of 50 µl and 100 µl for both honey brands. The *Ziziphus* honey showed highest zone of inhibition for, *S. pyogenes*, *P. aeruginosa*, and *E. coli* the zone of inhibition is (35 mm) while *S. aureus* showed (30 mm) zone of inhibition. At 50 µl the highest zone of inhibition was (25 mm) for *P. aeruginosa*. *Acacia* honey *S. pyogenes* showed highest zone of inhibition for both concentrations 50 µl and 100 µl which are (19 mm) and (32 mm), respectively. In contrast to our study Rajeswari and Mandal [30] studied two brands of honey: Manuka honey and Nilgiris honey were tested against pathogenic bacterial isolates. The zone of inhibition were determined against *E. coli*, *S. typhi*, *S. aureus* and *P. aeruginosa*, the zone of inhibition were (13 mm – 14 mm), and (17 mm – 19 mm), followed (20 mm – 21 mm) and (25 mm – 27 mm), respectively. A difference in results was due to variation in samples size, geographical difference and location from where honey was collected. Hussain et al. [31] conducted study on Maunka honey and local honey brand *E. coli*, *S. typhi*, *S. aureus* and *P. aeruginosa* zone of inhibition ranging from 13 mm, 14 mm and 16 mm, respectively. It was thus concluded that honey is an alternative to treat bacterial infection and helped to reduce the chance of emergent drugs resistance to a great extent.

MIC values are used to determine susceptibilities of bacteria to drugs and also to evaluate the activity of new antimicrobial agents. In current study four different concentration of honey were used, i.e., 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml for isolated *Staphylococcus aureus*, *Pseudomonas*, *Streptococcus pyogenes*, and *E. coli*. The MIC was determined by measuring optical density (OD) values at 610 mn using spectrophotometer [21]. Yilmaz [32] reported three different concentrations, i.e., 100 µl/mg, 50 µl/mg, 10 µl/mg in his findings. In *ziziphus* honey *S. aureus* growth was inhibited at 100 mg/ml and 75 mg/ml concentration and the MIC was 0.25 µl. In *Acacia* honey the growth of *S. aureus* was inhibited at 100 mg/ml concentration, the MIC was 0.5 µl. Similarly, for *Acacia* honey the growth of *P. aeruginosa* was inhibited at 100 mg/ml and 75 mg/ml, the lowest MIC is 1 µl. *P. aeruginosa* growth was inhibited in *Ziziphus* honey at 100 mg/ml, 75 mg/ml and the lowest MIC is 0.25 µl for both concentrations. *S. pyogenes* growth was inhibited at 100 mg/ml, 75 mg/ml concentrations and the lowest MIC is 0.25 µl for *Acacia* honey, whereas *Ziziphus* honey inhibited growth at 100 mg/ml, 75 mg/ml concentration on the lowest MIC at 0.5 µl, respectively. In *Ziziphus* honey *E. coli* growth was inhibited at 100 mg/ml concentration and the lowest MIC is 0.25 µl; however, *Acacia* honey inhibited the growth of *E. coli* at 100 mg/ml, 75 mg/ml concentration and the lowest MIC was 0.5 µl. Mandal et al. [33] determined the Antibacterial mechanism of honey toward pathogen *E. coli* (n = 5), *P. aeruginosa* (n = 5), *S. enterica* serovars *typhimurium* (n = 8). Also, MIC and PIC in their findings ranged from 1.75-3.0 and 3-3.5, respectively. The differences observed were due to the variation in sample size, geographical location and quality and type of honey used.

5. CONCLUSIONS

It was concluded from the present study that Surgical Site Infections (SSI) was among the highest prevalent diseases, affecting millions of people throughout the world. This can be achieved by optimal preoperative, intra-operative and post-operative patient care. This would be supported with proper infection control measures and balanced antibiotic policy. Infection by multidrug-resistant bacteria enhances the need for antibiotic policy guidelines in hospitals. Furthermore, it can be concluded that honey has effective antimicrobial properties against bacterial isolates. The honey showed excellent antibacterial activity against *Staphylococcus aureus*, *Pseudomonas spp*, *Streptococcus pyogenes*, *E. coli* at the Surgical Site Infections. From bioassay, it was revealed that the most potent antibiotics found against all the tested bacterial isolates showed maximum resistance against Gentamicin, Ciprofloxacin, and Amikacin.
Moreover, it is also established that honey would be effective against bacterial isolates responsible for SSI and must be used as an alternative of antibiotics because of resistance found in commercially available antibiotics. Furthermore, due to high antimicrobial activity of honey, further investigation is suggested in this regard as an alternative therapy for wound healing.

6. ETHICAL STATEMENT

The study was conducted in accordance with the declaration of Helsinki, and the protocol was approved by the Institutional Ethics Review Committee, Abasyn University Peshawar.

7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

8. REFERENCES


